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Mucor indicus isolated from the semiarid region of Brazil: a first record for South America

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Abstract—A specimen of *Mucor indicus* was isolated from a dung sample collected in the city of Sertânia in the Brazilian state of Pernambuco and its identity confirmed through LSU rDNA sequence analysis. The taxon is described and illustrated for the first time from South America. An identification key is provided for *Mucor* species from the northeast Brazilian semiarid region (Caatinga).

Keywords—lower fungi, phylogenetic analysis, *Mucoromycotina*, *Zygomycota*

Introduction

Mucor Fresen. (*Mucoraceae*) comprises species characterized by the formation of non-apophysate sporangia, producing simple or branched sporangiophores that emerge directly from the substrate. Few species have rhizoids and stolons are not produced (Benny 2014). This genus has a worldwide distribution and most of the species described are saprobes commonly isolated from a wide variety of substrates such as soil, stored grains, fruits, rotting vegetables, fleshy agarics, and herbivore dung (Viriato & Trufem 1985, Alves et al. 2002, Schoenlein-Crusius et al. 2006, Jacobs & Botha 2008, Santiago & Souza-Motta 2008, Santiago et al. 2013).

Although over 300 species have been cited in the literature (Jacobs & Botha 2008), the exact number of authentic taxa is unknown. According to Gherbawy et al. (2010), it is possible that the number of authentic species could range between 50 and 75. Schipper (1973, 1975, 1976, 1978) described 39 species, four varieties, and 11 forms of *Mucor*. Subsequently, 17 additional species have been proposed (Mehrotra & Mehrotra 1978, Mirza et al. 1979, Subrahmanyam 1983, Chen & Zheng 1986, Schipper 1989, Schipper & Samson 1994, Watanabe 1994, Zalar et al. 1997, Pei 2000, Alves et al. 2002, Jacobs & Botha 2008, Álvarez et al. 2011, Hermet et al. 2012, Madden et al. 2012).

Previous studies have treated *Mucor* as polyphyletic (O'Donnell et al. 2001, Kwasna et al. 2006, Jacobs & Botha 2008, Budziszewska & Piatkowska 2010, Álvarez et al. 2011). Based on the phylogeny of ITS and LSU rDNA regions of several mucoralean species, Walther et al. (2013) observed that some *Mucor* species with curved sporangiophores represent a natural grouping with *Backusella* Hesselt. & J.J. Ellis, and transferred nine *Mucor* species to *Backusella*.

The aim of the present study was to describe and illustrate *M. indicus* from dung samples collected in the city of Sertânia, Pernambuco, Brazil. An identification key for *Mucor* species from the semiarid region of Brazil is also provided.

Material & methods

Isolation and morphological & physiological identification of *M. indicus*

Samples of goat (*Capra hircus* Linnaeus) dung were collected at the Agronomic Institute of Pernambuco stations, located in the city of Sertânia (8°03′38″S 37°13′32″W) in the state of Pernambuco, 326 km away from Recife, Brazil. The mean annual temperature is 25°C, with rainfall of 635 mm per year. The area has a hyperxerophilic Caatinga vegetation. The samples were placed in clean plastic bags for transportation to the laboratory and then incubated in triplicate in moist chambers for 10 days. The plates were left on a bench at room temperature (28 ± 2°C) exposed to alternating light and dark periods. Fragments of mycelium were removed directly from Petri dishes under a Leica EZ4 stereomicroscope and transferred to Petri dishes with malt extract agar (MEA) (Benny 2008). The specimen was identified through observation of macroscopic (color, appearance, and diameter of the colony) and microscopic (e.g., shape and size of sporangiophores, columellae, sporangia, and sporangiospores) characteristics based on the descriptions of Schipper (1978). The thermotolerance of *M. indicus* was confirmed by growing it at 40°C for 7 days. The colony colors were designated according to Maerz & Paul (1950).

Molecular analysis

Cultures grown in test tubes containing malt extract were incubated at 28°C for 6 days to generate fungal biomass. The material was transferred to 2 mL microtubes with screw caps. Subsequently, 0.5 g acid-washed glass beads of two different diameters (150–212 µm and 425–600 µm, 1:1; Sigma, USA) were added to each tube. The material was crushed by stirring at high speed in a FastPrep homogenizer. The genomic DNA extraction procedure was conducted as described by Góes-Neto et al. (2005). The mycelium was washed with chloroform: isoamyl alcohol (24:1) and then homogenized

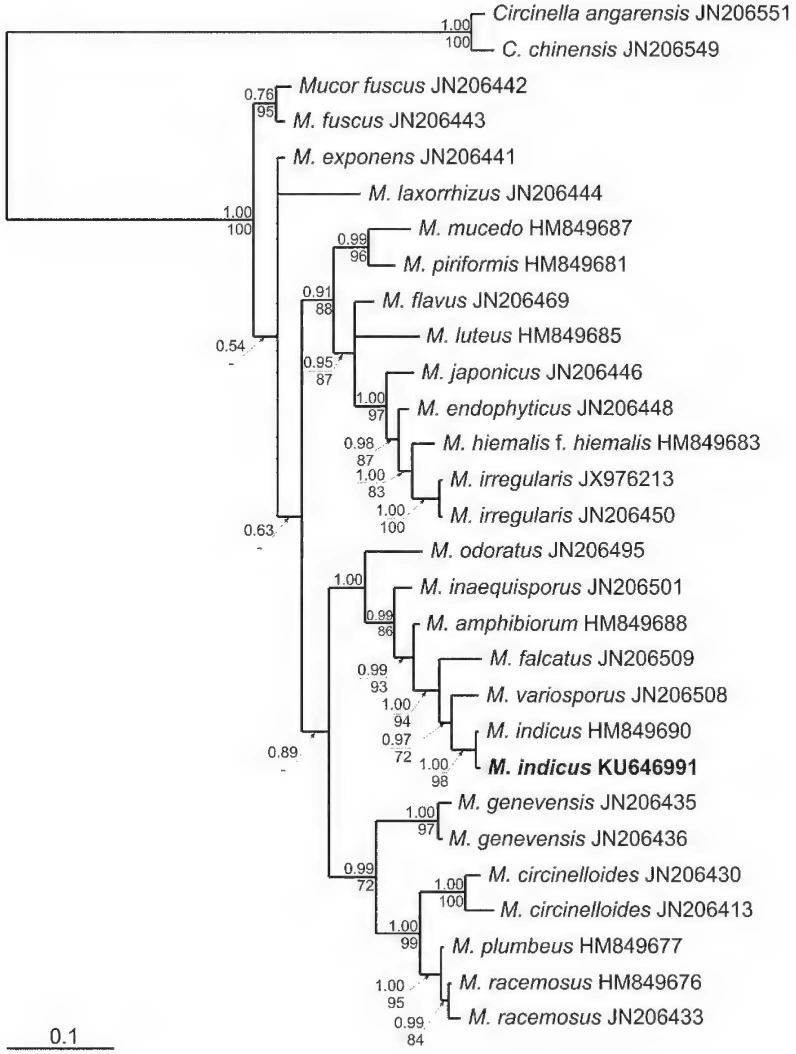

Fig. 1. Phylogenetic tree of *Mucor* species constructed using partial LSU rDNA sequences. *Circinella angarensis* and *C. chinensis* were used as outgroup. Sequences are labeled with their GenBank accession numbers. Branch support values are from Bayesian inference (above) and maximum likelihood (below) analyses. The sequence obtained in this study is in boldface.

in 2% cetyltrimethylammonium bromide (CTAB) buffer. The DNA was precipitated in chilled isopropanol, washed with 70% ethanol, and resuspended in 50 µL ultrapure water.

The primer pairs LR1/LSU2 were used to amplify a fragment of the large subunit (LSU) nuclear ribosomal DNA (rDNA) (van Tuinen et al. 1998, Santiago et al. 2014). The polymerase chain reactions were carried out as described by Oliveira et al. (2014). The newly obtained sequence was deposited in the National Center for Biotechnology Information GenBank database (accession number KU646991).

Phylogenetic reconstructions were obtained by analyzing the partial LSU rDNA sequences. The fungal sequences were aligned with ClustalX (Larkin et al. 2007) and edited with BioEdit (Hall 1999). Prior to the phylogenetic analysis, the optimal nucleotide substitution model was estimated using Topali 2.5 (Milne et al. 2004). Bayesian inference (two runs over 10^6 generations with a burn-in of 2500) analysis was performed with MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003), launched from Topali 2.5.

Results

Phylogenetic analysis

The Blastn analysis of the LSU sequences showed a 99% similarity between our Brazilian material (URM7222) and *M. indicus* (CBS 226.29; GenBank HM849690). Phylogenetic analysis of the sequence datasets (Fig. 1) showed that URM7222 formed a well-supported clade (BI 1.00/ML 98%) with the sequence of *M. indicus* (HM849690).

Taxonomy

Mucor indicus Lendn., Bull. Soc. Bot. Genève 21: 258 (1930). Fig. 2

Colony initially white and becoming deep yellowish (MP 11C1 – Amber White) with yellowish reverse (MP 11J6), reaching 9.4 cm in diam. and 9 mm in height after 4 days in MEA at 28°C. Sporangiophores erect (rarely circinate), repeatedly sympodially branched, with long branches, (4–)5–12.5(–14.5) µm diam., hyaline to yellowish, smooth-walled. Sporangia globose to subglobose, (22.5–)32.5–45(–50) µm diam., brownish, with diffluent wall. Columellae globose (27.5–)30–47.5(–50) µm, subglobose (25.5–)27.5–28.5 × 42.5–45(–47.5), or applanate (18–)20–21.25 × 27.5–30(–32.5) diam., grayish or yellowish brown, smooth-walled; collars small and infrequent. Sporangiospores hyaline, smooth-walled, often ellipsoid (3.75–)4–6.25 × 3–6.5(–7.5) µm and subglobose 4–6(–7.5) µm in diam. Chlamydospores present. Zygosporangia not observed. Growth reasonably good at 40°C (7.6 cm in 168 hours) in MEA with rare sporangiophore production and poor sporulation.

Habitat: Recorded on goat dung in the semiarid region of Pernambuco, northeastern Brazil.

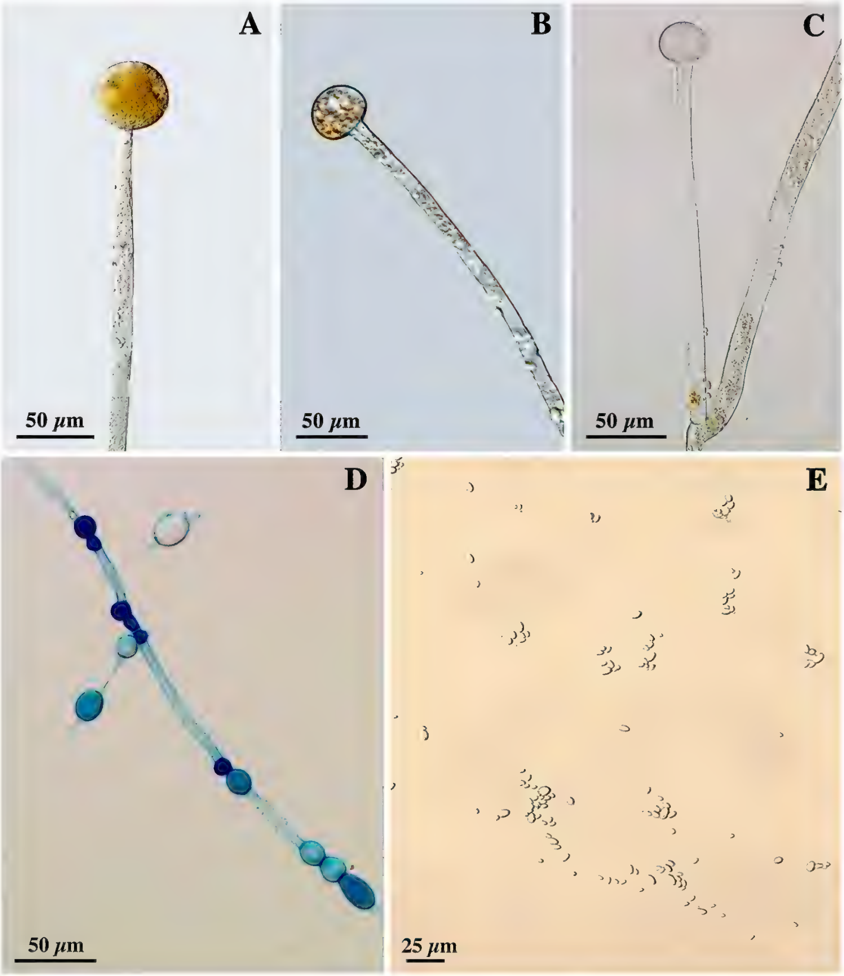

Fig. 2. *Mucor indicus* (URM 7222): A. Sporangiophore simple with sporangia; B. Sporangiophore simple with columella; C. Sporangiophore branch with columella; D. Chlamydospores; E. Sporangiospores.

> Specimen examined: **BRAZIL**, **Pernambuco**, Sertânia: Instituto Agronômico de Pernambuco (IPA), 8°03′38″S 37°13′32″W, from goat dung, 5.XI.2014, leg. C.A.F de Souza (URM 7222).

Geographic distribution: India (Lendner 1930, Abe et al. 2004), Poland (Szwedek-Trzaska & Glowacka 2011), Spain (Luna et al. 1986, García-Pantaleón et al. 1992, Herrero et al. 1996), USA (Oliver et al. 1996, Sobel 2001, Álvarez et al. 2009), and Vietnam (Lee & Fujio 1999). Our Brazilian collection represents the first report from South America.

Discussion

The morphological characteristics of *M. indicus* reported here show a close similarity with the description by Schipper (1978), although there are differences in the diameter of sporangiospores and columellae. Sporangiospore sizes reported in the literature (5.4–5.7 × 4.4 µm; Schipper 1978) are smaller than our maximum measurements, whereas columellae were larger (≤56 × 53 µm; Schipper 1978) than observed in our isolate. *Mucor indicus* strains may exhibit morphological similarities to *M. circinelloides* Tiegh. (Schipper 1978). However, *M. indicus* lacks the abundant short branches reported for *M. circinelloides* and its predominantly globose columellae differ from the obovoid type found in *M. circinelloides*. Moreover, the thermotolerance inherent to *M. indicus* is not found in *M. circinelloides* (Schipper 1978). Walther et al. (2013) have reported that although some *M. indicus* cultures morphologically resemble *M. circinelloides*, there are clear genetic differences between these two species.

The LSU phylogenetic tree (Fig. 1) suggests that *M. indicus* and *M. variosporus* Schipper are genetically closely related. Morphologically, however, *M. indicus* colonies are lower than those of *M. variosporus*, which can reach 20 mm in height (Schipper 1978). Differences in sporangiophore diameters have also been observed, with *M. variosporus* producing larger (14(–23) µm) sporangiophores. Finally, *M. variosporus* sporangia are yellow and larger (≤150 µm diam.) than those of *M. indicus,* which are brownish and smaller.

This manuscript reports the first occurrence of *M. indicus* in South America, thereby expanding our knowledge of mucoralean distribution. In the semiarid region of Brazil (Caatinga), other *Mucor* species have been isolated, such as *M. circinelloides* Tiegh. f. *circinelloides, M. hiemalis* Wehmer, *M. luteus* Linnem. ex Wrzosek., *M. prayagensis* B.S. Mehrotra & Nand ex Schipper, *M. racemosus* Fresen., and *M. subtilissimus* Oudem. (Santiago 2016). During a recent survey of *Mucorales* from other Brazilian semiarid regions, *M. circinelloides* Tiegh. f. *griseocyanus* (Hagem) Schipper, *M. circinelloides* Tiegh. f. *janssenii* (Lendn.) Schipper, *M. circinelloides* Tiegh. f. *lusitanicus* (Bruderl.) Schipper, *M. racemosus* Fresen. f. *racemosus,* and *M. variosporus* Schipper were also isolated (unpublished data).

Identification key for *Mucor* taxa isolated from the semiarid region of Brazil

1. Sporangiospores regular in form and size 2
1. Sporangiospores variable in size and shape: globose, ovoid, cylindrical, and fusiform . . . . *M. variosporus*

2. Sporangiophores unbranched or slightly sympodially branched	3
2. Sporangiophores repeatedly branched	6
3. Columellae globose to subglobose or globose to applanate; sporangiospores ≤15 µm long with or without a granule	4
3. Columellae spherical or ellipsoidal with a truncated base; sporangiospores ≤8 or 10 µm long	5
4. Sporangiophores unbranched; columellae subglobose to applanate; sporangiospores ellipsoidal or flattened on one side	*M. prayagensis*
4. Sporangiophores sparsely branched; columellae globose to subglobose (rarely ellipsoidal); sporangiospores ellipsoidal with a granule at each end	*M. subtilissimus*
5. Columellae ellipsoidal with a truncate base; sporangiospores ellipsoid, sometimes flattened on one side, 2.5–10 × 2–7.5 µm	*M. hiemalis*
5. Columellae globose; sporangiospores long-elliptical and fusiform 2.5–8.1 × 1–5 µm	*M. luteus*
6. Absence of growth at 40°C	7
6. Good growth at 40°C	*M. indicus*
7. Sporangiophores sympodially branched; chlamydospores present or not, when present not abundant and never formed in sporangiophores or columellae	8
7. Sporangiophores sympodially and monopodially branched; chlamydospores abundant, some formed in sporangiophores and columellae	*M. racemosus* f. *racemosus*
8. Colonies initially white, then becoming gray; sporangiophores ≤10 µm in diam.; sporangia brownish-black	9
8. Colonies initially yellow than turning brownish; sporangiophores ≤17 µm in diam.; sporangia gray-brownish	10
9. Sporangiospores ellipsoid	*M. circinelloides* f. *griseocyanus*
9. Sporangiospores globose to slightly subglobose	*M. circinelloides* f. *janssenii*
10. Columellae obovoid; sporangiospores ellipsoid	*M. circinelloides* f. *circinelloides*
10. Columellae globose; sporangiospores ellipsoid, some irregular in shape	*M. circinelloides* f. *lusitanicus*

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